

## **Date of Annual Report: 05/09/2013**

### **Report Information:**

- Annual Meeting Dates: 02/27/13
- Period the Report Covers: 10/2011 to 09/2012

### **Project participants:**

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- Dobson, Stephen (email) Kentucky, University of Kentucky (Present)
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- Fonseca, Dina (email), New Jersey, Rutgers University (Present)
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### **Brief Summary of Minutes of Annual Meeting:**

The annual meeting of the Multi-State Project NE1043, Biology, Ecology and Management of Emerging Disease Vectors was held on February 27, 2013, at the Trump Taj Mahal hotel in Atlantic City, New Jersey. The meeting was held in conjunction with the annual conference of the American Mosquito Control Association.

The following were in attendance: Walker (MI), Armstrong (CT), Dobson (KY), Federici (CA), Wirth (CA), Leisnham (MD), Gaugler (NJ), Fonseca (NJ), Kline (ARS-Gainesville),.

Dr. Ned Walker (Chair) reviewed the agenda, local arrangements and format for the meeting. He circulated annual reports and noted that reports were submitted from 11 participating agencies

(CT, MD, MN, NJ, NY, OK, WI, MI, NE, KY, USDA-Gainesville). The agenda included an overview, a session of individual project reports before, during, and after a buffet lunch, and a general session on status of the project and project renewal opportunities.

Dr. Kirby Stafford (Administrative Advisor) was unable to be present, however, he provided in writing a notification of the potential for renewal of the project and that the date for renewal was fast approaching. He further provided the following guidance.

“Ideally, a request to rewrite would be submitted at this meeting, but it won’t be accepted without a mid-term review and there is little time between February 27 and March 11, especially with me gone for three days the first week in March. A request can be submitted at the summer meeting and still keep on track, but in this case it might help to start work on a proposal ahead of time. The group needs to seriously think about whether to try and renew as a multi-state research project or as a coordinating committee. From what I can tell, the group is really functioning as a coordinating committee (CC) as your research activities are not jointly planned and multistate. However, while a CC will cover travel and getting together as a group, I believe it won’t cover research expenses. I have to double check that. We haven’t used CCs much in the northeast region. [They are as follows:] Multistate Research Coordinating Committees (CC) and Education/Extension and Research Activity (ERA): The membership of a CC or an ERA is made up of an AA, CSREES representative, scientists, and as applicable, extension specialists and/or extension agents. A CC or ERA provides opportunity for scientists, specialists, and others to work cooperatively to solve problems that concern more than one state, share research data, and coordinate research and other types of activities. This is presently one of the most common mechanisms for functionally integrated activities such as the regional IPM programs.

[By contrast,] The membership of a Multistate Research Project is called the technical committee, and is made up of SAES scientists, an AA, CSREES representative, other public and private sector scientists, and as applicable, extension specialists and/or extension agents. This type of activity involves cooperative, jointly planned research employing multidisciplinary approaches in which a SAES, working with other SAESs, the Agricultural Research Service (ARS), or a college or university, cooperates to solve problems that concern more than one state and usually more than one region. In addition, the following must be demonstrated in the project proposal: 1. The objectives are clearly focused. 2. Each participant listed has direct involvement in the accomplishment of the stated objectives. 3. The project is multistate and multidisciplinary. 4. The project proposal has been peer-reviewed. 5. The proposed project is oriented toward accomplishment of specific outcomes and impacts and based on priorities developed from stakeholder input. 6. The project is responsive to CSREES goals.

This guidance statement was read aloud by Dr. Walker, followed by discussion. There was no consensus regarding continuation of the NE

Brief research updates were given by each cooperator with active discussion ensuing. A group photo was taken.

**Accomplishments:**

Objective 1. Strengthen basic and applied research on the mosquito, pathogen, hosts, and environmental factors that influence disease emergence.

To determine whether West Nile virus (WNV) overwinters locally or is reseeded annually, we examined the patterns of viral lineage persistence and replacement in CT over 10 consecutive transmission seasons by phylogenetic analysis. We compared the full protein coding sequence among WNV isolates to search for evidence of convergent and adaptive evolution. Viruses sampled from CT segregated into a number of well-supported subclades by year of isolation with few clades persisting  $\geq 2$  years. Similar viral strains were dispersed in different locations across the state and divergent strains appeared within a single location during a single transmission season, implying widespread movement and rapid colonization of virus. Numerous amino acid substitutions arose in the population but only one change, V $\rightarrow$ A at position 159 of the envelope protein, became permanently fixed. Several instances of parallel evolution were identified in independent lineages, including one amino acid change in the NS4A protein that appears to be positively selected. Results suggest that annual reemergence of WNV is driven by both reintroduction and local-overwintering of virus. Despite ongoing diversification of WNV, most amino acid variants occurred at low frequencies and were transient in the virus population (CT).

*Cx. tarsalis* is a superior horizontal and vertical vector of WNV compared with *Cx. salinarius*. *Cx. salinarius* transmitted WNV genotype NY99 (CT 2741-99 strain) horizontally to suckling mice at significantly lower rates than *Cx. tarsalis* and *Cx. salinarius* transmitted WNV genotype NY99 to offspring at a lower vertical transmission infection rate than *Cx. tarsalis*. *Cx. tarsalis* transmitted WNV genotypes NY99 and WN02 (CT S0084-08 strain) with equal efficiency. Daily percent horizontal transmission of genotype NY99 by *Cx. tarsalis* -infected per os and by intra-thoracic infection was not significantly different from daily transmission of genotype WN02. Findings do not support the previously published hypothesis that genotype NY99 was replaced in the New World by WN02 because of a shorter extrinsic incubation of WN02 (CT).

A survey of mosquito larvae infected with microsporidia was conducted from 2005-08 in the Tomsk, Kemerovo and Novosibirsk regions of western Siberia, Russia. Twenty-one morphologically and genetically unique species of microsporidia were isolated from 9 species of mosquitoes including 14 new species of *Amblyospora*, a new genus and species, *Novothelohania ovalae* and 6 species of *Amblyospora*, *Parathelohania* and *Trichoctosporea* from which gene sequences had not been previously obtained. Detailed ultrastructure of meiospores revealed unique cytological features associated with the length, arrangement and ratio of broad to narrow coils of the polar filament, comparative thickness of the exospore and endospore, and overall size of each species reaffirming their value in distinguishing taxonomic relationships. SSU rDNA sequences were unique when compared with GenBank entries. Phylogenetic trees yielded similar topologies with a high degree of congruence between parasite and host at the generic level. Species that parasitize *Aedes/Ochlerotatus* and *Culex* mosquitoes segregate into distinct monophyletic groupings mirroring their host phylogeny, while species from *Anopheles* mosquitoes group as a sister clade basal to the entire group of mosquito-parasitic microsporidia as their *Anopheles* hosts cluster as a sister clade to the entire group of culicine mosquitoes. This provides strong evidence for host-parasite coevolution by descent at the generic level and limited host lineage switching between unrelated taxa. Among parasites of *Aedes/Ochlerotatus* and *Anopheles* mosquitoes, we found several instances where a single mosquito species serves as a

host for two or more related species of microsporidia, an observation consistent with host switching and independent parasite speciation. Among the microsporidian parasites of *Culex* mosquitoes, we found only one parasite per host indicating a higher degree of host specificity and less host switching among parasites of this genus. Findings suggest a degree of host-parasite co-speciation with host switching occurring occasionally when the normal host is unavailable in the aquatic ecosystem. Frequency of host switching seems to be occurring in proportion to host relatedness and does not cross generic boundaries in this system (CT).

The spread of exotic mosquito species into new environments can introduce shifts in mosquito populations and potentially alter public health risks to mosquito-borne diseases. The successful establishment of exotic species may occur due to their competitive advantage over other cohabitating species. We hypothesized that the recently introduced exotic mosquito *Ae. japonicus* would be a more effective competitor than *Ae. atropalpus* and *Ae. triseriatus*, and an equal competitor to *Cx. pipiens* based on larval abundance data within tire habitats. Impacts of competition were measured using the larval developmental rate and survival of larvae, adult mortality, wing length, and sex ratio. We found that intraspecific competition acted strongest against *Ae. japonicus* versus the other three resident mosquito species by delaying larval development and increasing adult mortality. Interspecific competition was generally weak and significant main effects were only detected for species and density. Results show that larval competition between *Ae. japonicus* and the three resident species was weak when present, indicating that other ecological or behavioral factors may be influencing the invasion success for *Ae. japonicus* in North America (CT).

Life-table experiments were conducted to test for differences in the adult survival and reproductive schedules of *Ae. albopictus* females from three populations from the northern and southern extremes of the species distribution in North America. There were consistent differences between northern and southern populations in incidence of photoperiodically-induced egg diapause. Under short daylength, diapause eggs constituted twice the proportion of total viable eggs from northern females than southern females. There were no consistent differences between northern and southern populations in resource allocation between reproduction and maintenance, reproduction over time, and reproductive investment among offspring, and no apparent trade-offs between diapause incidence with reproduction or longevity. Results suggest that the main response of North American *Ae. albopictus* to unfavorable winter climates is via the life history strategy of producing diapausing eggs, rather than quantitative variation in reproduction, and that there are no detectable costs to adult survival (MD).

A study was conducted to characterize mosquito communities among different habitats in historically ditched tidal salt marshes and adjacent wooded areas in a wetland management area on the Maryland Delmarva Peninsula. Wooded habitats had more total mosquitoes, were more frequently occupied by mosquitoes, and had higher densities of mosquitoes. The majority of larvae at the control site were *Ae. sollicitans* in marsh pannes while *Cx. salinarius*, *An. bradleyi*, *Ae. cantator*, and *Ae. sollicitans* were collected in high numbers from ditches at the experimental site. We found a difference in the proportion of marsh pannes occupied by *Ae. sollicitans* but not total mosquitoes sampled 45 days after spring tide events than on other occasions (MD).

Carbon-based secondary or structural plant compounds usually increase when they are exposed to elevated atmospheric CO<sub>2</sub>, and may differentially affect container mosquito species that feed on litter that fall into water-filled containers. Competition between *Ae. albopictus* and *Ae. triseriatus* was tested in laboratory microcosms provisioned with litter grown in either elevated (1,000 ppm) or ambient CO<sub>2</sub> (385 ppm). In additional microcosms, single-species cohorts of either *Ae. albopictus* or *Ae. triseriatus* were provided with increasing amounts of one of the two litter types or concentrations of commercial tannic acid to test potential toxicity effects of leaf-derived tannins. We found no differences in mosquito competition between litter types, with *Ae. albopictus* consistently superior to *Ae. triseriatus*. Single-species microcosms provisioned with elevated leaf litter had slower male development time than microcosms with ambient (lower-tannin) litter. *Ae. albopictus* and *Ae. triseriatus* also experienced similarly higher mortality and slower development with increasing commercial tannic acid concentrations. These results suggest that possible litter grown in high CO<sub>2</sub> conditions may suppress mosquito production by increasing the toxicity of leaf litter, but that it would not affect population-level competition between *Ae. albopictus* and *Ae. triseriatus* (MD).

We conducted yard surveys for mosquito larval habitats paired with knowledge, attitude, and practice questionnaires administered to residents among six neighborhoods in Washington DC that vary in socioeconomic status. Household income was related to overall knowledge and attitudes of residents towards mosquitoes, but that knowledge and attitudes were not related with source reduction as measured through questionnaire responses or actual numbers of water-holding containers in their yard. Numbers of water-holding containers was strongly positively related to exposure to *Ae. albopictus*, *Cx. pipiens* and pupae but not related to whether or not households practiced source reduction. Households that reported practicing source reduction had lower *Cx. pipiens* infestation and abundances but no difference in *Ae. albopictus* or pupal infestation or abundance. Results suggest large households are a greater source of vector mosquitoes and should be targeted in education campaigns, and that self-perceived source reduction of residents may not reduce urban mosquito habitat (MD).

The risk presented to humans by circulation of WNV in the *Culex* bird enzootic cycle established in metropolitan areas of the upper Midwest was continued. *Culex* Flavivirus was positively associated with WNV infection in a case-control study of mosquito pools collected from an endemic focus of WNV transmission in Chicago. This study provides direct evidence that insect-specific flaviviruses may influence human health risk by enhancing WNV infection in mosquitoes that are epidemic bridge vectors to human hosts (MI).

Analysis of molecular genetic diversity in 9 marker regions of 5 genes within the bacteriophage WO region of the *Wolbachia pipentis* genome revealed high diversity of wPip in the population of *Cx. pipiens* sampled in metropolitan Chicago, IL. *Wolbachia* endosymbionts may modulate propensity to infection with this virus and may affect population structure of their mosquito hosts. Multiple regression showed that markers associated with gene Gp2d were significantly associated with ancestry of individuals to form *molestus* or form *pipiens*, as determined by prior microsatellite allele frequency analysis. Data suggest that certain wPip molecular genetic types are associated with genetic substructuring in the *Cx. pipiens* complex of Chicago, and that the association extends to host preference. The influence of host diversity on multi-host pathogen persistence and transmission is confounded by the large number of species and complex

biological interactions that comprise the transmission system. Although diversity and composition of host communities have been hypothesized to affect intensity of transmission, the effect of host community structure on the transmission of vector-borne pathogens remains largely unknown. Results suggest that host community measures, such as species diversity, may not be useful indicators of transmission risk at fine spatial scales in vector-borne disease systems (MI).

*Culex* mosquitoes carry an intracellular symbiont, *W. pipiens*, a bacterium which can potentially be manipulated to control vector populations by spreading a reproductive distortion called cytoplasmic incompatibility. We obtained a *Cx. pipiens* colony from Ohio State University and, for comparative purposes, have developed a sister colony cured of *Wolbachia* infection. By comparing protein bands from dissected testes, we identified proteins produced in response to *Wolbachia* infection. Efforts are underway to clone two *Wolbachia* DNA binding proteins that are implicated in the reproductive distortion known as cytoplasmic incompatibility. Parallel investigations with *Wolbachia*-infected cell lines are used to identify *Wolbachia*-inducible protein expression in undifferentiated cells that do not engage in gamete production. We are investigating methods to modulate expression of these proteins by testing effects of specific components, including the DNA synthesis inhibitor mimosine and the mitochondrial inhibitor, paraquat, in the culture medium. We are finalizing methodology for quantifying *Wolbachia* infections using fluorescence activated cell sorting (MN).

*Ae. japonicus*, which was first collected during summer 2010, was again collected during summer 2011 in eastern NE. Specimens were collected using CDC-light traps baited with carbon dioxide. Mosquitoes were collected in four sites within Lincoln, NE. The importance of *Ae. japonicus* to vector disease ecology and epidemiology in eastern NE is not known. (NE).

Using a panel of seven microsatellite loci, we confirmed the existence of two abundant genetic forms in specimens originally collected in 1999-2000 that matches the disjunctive distribution of mitochondrial haplotypes. To examine the distribution of the two genetic types across PA we created a fine scale genetic map of *Ae. japonicus* using specimens collected from 54 Pennsylvania counties in 2002-2003. We also made direct comparisons between collections in 1999-2000 and new collections made in 2004-2005 obtained from the same areas in the northeastern US. We observed that the strong association between mtDNA haplotype and microsatellite signature seen in 1999-2000 had weakened significantly by 2002 across PA, a trend continued to some extent in 2004-2005 in PA, NJ, and NY, indicating that once easily distinguishable separate introductions are merging. The two expanding genetic forms create a complex correlation between spatial and genetic distances. The existence of multiple introductions would be obscured without sampling early and across time with highly polymorphic molecular markers. Results provide a high resolution analysis of the spatial and temporal dynamics of a newly introduced disease vector and argue that successive introductions may be a common pattern for invasive mosquitoes (NJ)

We documented the potential importance of eastern red cedar, *Juniperus virginiana* leaves and fruit as a larval resource for the invasive *Ae. albopictus* in OK. We examined how different leaf species commonly found in FL interact to affect competition within and between *Ae. albopictus*

and *Aedes aegypti*. We have also continued investigations into the effects of temperature and food on growth in larval mosquitoes (OK).

Laboratory and field tests of potential biological control agents were conducted for control of *Cx. pipiens*. The key goals of this project are to test locally adapted fathead minnows and copepods for control of *Cx. pipiens/restuans*. Fish are used in aboveground breeding sites while copepods are used in the underground catch basin system. Fish were not released in 2011 because of extended dry conditions which limited the extent of targeted wet sites and the occurrence of larvae in those sites. Belowground sites did begin to generate toward the end of the season and these were used for trials of copepod releases. We tested the efficacy of copepods as predators of *Culex* or *Anopheles* larvae. *Acanthocyclops trajani* and *Macrocyclus albidus* were tested against *An. stephensi* and *Cx. pipiens*. *M. albidus* females killed 50% of *Culex* larvae within 24 hr while *A. trajani* killed only 35% of larvae. Results for *An. stephensi* were similar. We tested whether releases of *M. albidus* could affect larval *Culex* in catch basins (WI).

We released *M. albidus*, into storm drain catch basins in Madison, WI at the beginning of August. We monitored these sites along with untreated control sites until the end of September. The catch basins were sampled every week using a mosquito dipper, and immature mosquitoes were collected, counted, and reared to adulthood for identification of the species. All adult mosquitoes collected were identified as *Cx. pipiens*. Copepods were collected during the sampling and identified as *M. albidus* or *A. vernalis* or inconsequential species. Catch basins were infested with mosquito larvae. *M. albidus* failed to establish in all but one location. This single location, however, maintained and multiplied the number of *M. albidus* with many being collected every week. *M. albidus* colonized an adjacent catch basin after approximately 1 month and their numbers continued to increase throughout the survey. Citywide water main flushing after our introductions took place may have resulted in the flushing of *M. albidus* from the test sites. Heavy rainfall events may have also flushed these copepods from their new habitats on more than one occasion. The *A. vernalis* colony was created from the catch basin samples and these copepods were frequently found in our sites. They appear better adapted to the catch basin environment than *M. albidus* (WI).

Objective 2. Use knowledge of mosquito, pathogen, vertebrate reservoir, and environment interactions to enhance ability to predict conditions leading to disease. Seasonal epizootics of vector-borne pathogens infecting multiple species are ecologically complex and difficult to forecast. Pathogen transmission potential within the host community is determined by the relative abilities of host species to maintain and transmit the pathogen and by ecological factors influencing contact rates between hosts and vectors. Increasing evidence of strong feeding preferences by a number of vectors suggests that the host community experienced by the pathogen may be very different from the local host community. We developed an empirically informed transmission model for WNV in 4 sites using *Cx. pipiens* and preferred and non-preferred avian hosts. We measured strong feeding preferences for American robins (*Turdus migratorius*) by *Cx. pipiens*, quantified as the proportion of *Cx. pipiens* blood meals from robins in relation to their abundance (feeding index). The model accurately predicted WNV prevalence in *Cx. pipiens* at 3 of 4 sites. Sensitivity analysis revealed feeding preference was the most influential parameter on intensity and timing of peak WNV infection in *Cx. pipiens* and a threshold feeding index for transmission was identified. Findings indicate host preference

induced contact heterogeneity is a key mediator of vector-borne pathogen epizootics in multi-species host communities, and should be incorporated into multi-host transmission models (CT).

We studied the impacts of landscape heterogeneity and climatic variability on the richness and diversity patterns of mosquitoes and on the abundance and WNV infection rate of *Cx. pipiens*. Heterogeneity in the landscape was the best predictor of both mosquito species richness and diversity, with the most heterogeneous landscapes harboring the largest number of species. In general there were no changes in species richness over the years that could be associated with weather patterns and climatic variability. Although MIR was independent of mosquito diversity, it was associated with overall mosquito abundance, which had a convex association with species richness (i.e. abundance increases to a point after which it decreases as function of species richness). Results highlight the importance of considering dominant vector species as part of a community of vectors, whose biodiversity patterns can directly or indirectly impact the risk of infectious disease transmission (MI).

We examined vector competence of *Cx. pipiens* and *Cx. restuans* mosquitoes for WNV collected over 2 years from 16 local sites across 2 states to measure spatial and temporal variation in vector competence. We found extreme variation in vector competence with 0-52% of mosquitoes transmitting WNV at a single site between different sampling periods, and similar variation across populations. We also found that vector competence within a smaller geographic range (counties within a state) tended to vary somewhat synchronously, supporting environmental or population genetic drivers of variability in vector competence. In support of genetic influences, we found significant genetic variance between mosquitoes that became infected with WNV after feeding on infected blood, and those that did not. These results highlight the spatio-temporal variability in vector competence in mosquito populations and indicate that temporal variability may be influenced by local processes, such as population genetics and environmental factors. It is important that consideration of vector competence in risk estimates take this variability into account when interpreting any single measurement (NJ).

Chikungunya (CHIK) fever is a mosquito-borne viral infection that has started to invade temperate countries showing a surprising potential for geographic expansion. We developed a model for disease introduction based on virus introduction by one individual. Our study combines a climate-based mosquito population dynamics stochastic model with an epidemiological model to identify temporal windows that have epidemic risk. We ran this model with temperature data from different US locations (combined with strain-specific *Ae. albopictus* development and CHIK virus transmission rates) to study the geographic sensitivity of epidemic potential. We found that in locations with marked seasonal variation in temperature there also was a season of epidemic risk matching the period of the year in which mosquito populations survive and grow. In these locations controlling mosquito population sizes might be an efficient strategy. But, in other locations where the temperature is enough for mosquito development all year around the epidemic risk is high and (practically) constant. In these locations, mosquito population control alone might not be an efficient strategy control and other strategies should be implemented to complement it. Results suggest that in the event of an introduction and establishment of CHIK in the US endemic and epidemic regions would emerge initially, primarily defined by environmental factors controlling annual mosquito population cycles. These regions should be identified to plan different intervention measures. In addition, reducing

vector:human ratios can lower the probability and magnitude of outbreaks for regions with strongly marked seasonal temperature patterns (NY).

We continued extending our work on the landscape ecology of mosquito assemblages, transmission of dog heartworm, larval ecology, and mosquito oviposition behavior. We found highly significant segregation of mosquito species assemblages at scales of less than 100m, with different groups of species found in pasture, deciduous forest, and pasture invaded with eastern red cedar *J. virginiana*. We found significantly different rates of dog heartworm in vectors in urban compared to rural sites, and variable rates of dog heartworm and other parasites in coyotes across Oklahoma (OK).

Objective 3. Develop strategies to control mosquito vectors.

Catch basins are a major source of *Cx. pipiens*, *Cx. restuans*, and *Ae. japonicus* in northeastern U.S. VectoBac® CG (ai, *Bacillus thuringiensis israelensis* (Bti), VectoLex® CG (ai, *Bacillus sphaericus* (Bs), and VectoBac® 12AS (ai, Bti), each applied at maximum label rates (VectoBac CG, 1.8g per catch basin, VectoLex CG, 1.8g per catch basin, and VectoBac 12 AS, 0.193 ml per catch basin) significantly reduced numbers of larvae for one week. The dosages on the labels for treatment of mosquito larvae in catch basins, where mosquito breeding is continuous, are not adequate for providing long-term control in northeastern US without the need for frequent retreatment. When applied at 3 times the maximum label rate, VectoLex CG, VectoBac 12AS, and VectoBac CG significantly reduced numbers of larvae for 5, 4, and 2 weeks, respectively. A single application of VectoMax™ WSP (ai, Bti + Bs) per catch basin significantly reduced numbers of 3rd and 4th instar larvae and healthy pupae in catch basins but numbers of 3rd and 4th instars in treated catch basins at 21 days post-treatment had increased to 40% of the numbers in untreated catch basins. A second treatment of 1 pouch per catch basin reduced the numbers of 3rd and 4th instars and healthy pupae to near zero. VectoMax applied as 1 pouch per catch basin on July 1 and again on August 18 significantly reduced numbers of healthy pupae throughout the summer until the end of September. A second application of VectoMax to catch basins is likely needed during summer, when rainfall averages 13.7 inches during June through September, to keep numbers of *Culex* and *Ae. japonicus* significantly reduced to lower risk of human exposure to WNV. The application of one Natular™ XRT tablet, each weighing approximately 40.5 g (ai, 6.25% spinosad), to individual catch basins significantly reduced total numbers of larvae for 5 weeks (CT).

Funded by the Area-wide management of the Asian tiger mosquito (USDA-ARS) we have tested, optimized, and shared with the mosquito control community multiple methodologies to control *Ae. albopictus*. We have made multiple presentations at AMCA, ESA, NJMCA, ASTMH (NJ, ARS-Gainesville, FL).

A broad based approach to new toxicant discovery includes screening of conventional chemical libraries and structure activity relationship analysis, evaluation of registered compounds for mosquitocidal activity and bioassays of compounds derived from natural products. In addition, we are exploring gene silencing using RNAi technology as a method to knock down critical proteins in mosquitoes with possible applications for control (ARS-Gainesville, FL).

Studies on mosquito pathogenic viruses and microsporidia are focused on pathogen/host interactions. A cell culture system for the mosquito iridescent IIV-3 has been developed for conducting functional genomic investigations. Studies investigating the immune response in mosquitoes challenged with a baculovirus (CuniNPV) have found that a reaper ortholog (mx) is induced but apoptosis is blocked in permissive hosts by an unknown mechanism. Complete genomic sequences for three mosquito microsporidia are nearly completed. Sequencing of the genomes of *Edhazardia aedis* and *Vavria culicis* are completed and annotation is in the final stages. *Anncallia algerae* sequencing is underway. The annotation of all three species is expected to be completed in late 2012 (ARS-Gainesville, FL).

Objective 4. Enhance surveillance technologies for mosquitoes and mosquito-borne pathogens.

Mosquitoes transmit a number of distinct viruses including important human pathogens such as WNV, dengue virus, and chikungunya virus. Many of these viruses have intensified in their endemic ranges and expanded to new territories, necessitating effective surveillance and control programs to respond to these threats. One strategy to monitor virus activity involves collecting large numbers of mosquitoes from endemic sites and testing them for viral infection. We describe how to handle, process, and screen field-collected mosquitoes for infectious virus by Vero cell culture assay. Mosquitoes are sorted by trap location and species, and grouped into pools containing 50 individuals. Pooled specimens are homogenized in buffered saline using a mixer-mill and the aqueous phase is inoculated onto confluent Vero cell cultures. Cell cultures are monitored for cytopathic effect from days 3-7 post-inoculation and any viruses grown in cell culture are identified by the appropriate diagnostic assays. By utilizing this approach, we have isolated 9 different viruses from mosquitoes collected in CT, and among these, 5 are known to cause human disease. Three of these viruses (WNV Potosi virus, and La Crosse virus) represent new records for North America or the New England region since 1999. The ability to detect a wide diversity of viruses is critical to monitoring both established and newly emerging viruses in the mosquito population (CT).

In rural areas, dry ice availability can be a problem. Octenol has a stable shelf life, does not need to be refrigerated and is commercially available. Last year, we compared a widely-available octenol gel product (Nosquito octenol lure 24%) versus dry ice in CDC light traps. The findings demonstrated that overall mosquito and *Culex* spp. capture was not statistically significant using octenol and *Culex* spp. and total mosquito captures were significantly higher using dry ice compared to the octenol lure. We also continued studies assessing the use of octenol as a chemoattractant. Our objective was to assess if different concentrations and forms of the compound influence *Culex* spp. collections. Treatments include single vs. multiple octenol gels, octenol gel vs. liquid octenol, and low vs. high diffusion of liquid octenol (NE).

We used two mitochondrial loci (nicotinamide adenine dinucleotide dehydrogenase subunit 4 and cytochrome oxidase II) and a nuclear locus (28S-D2 spacer) for a total of 1337 bp to evaluate the relationships among the four subspecies of *Ae. japonicus* which was recently introduced into the US and has been expanding rapidly. We included in our analysis a morphologically very closely related species, *Ae. koreicus*, as well as 3 more distantly related species: *Ae. togoi*, *Ae. hatorii*, and *Ae. vexans*. We found that the 4 subspecies in the *Ae. japonicus* complex are genetically distinct but form a monophyletic group that includes *Ae.*

koreicus, suggesting the need for a taxonomic reconsideration of the group. We found the 2 southern subspecies are more closely related to each other than to any of the remaining subspecies or to *Ae. koreicus* and may indicate an ancient north-south split of the lineage. Considering the overlap between *Ae. j. japonicus* and *Ae. koreicus*, but the stronger association between *Ae. koreicus* and humans, we are surprised it also has not expanded from its original range. As a proactive reaction to this possibility, we designed and tested a DNA-based rapid assay to differentiate *Ae. koreicus* from some of the species with which it may be confused in the US. Belgian and Italian researchers have used our rapid assay to conclude that *Ae. koreicus* has expanded to Belgium and Italy. These *Aedes* are putative vectors of several important viral encephalitides (NJ).

We developed new methodology to identify the bloodmeals of *Ae. albopictus* using blocking primers to avoid nuclear copies and developed a new high throughput qPCR methodology to identify large numbers of container *Aedes* eggs (NJ).

We examined the importance of physical size characteristics of larval habitats in oviposition choice in *Ae. albopictus*. This information can be applied to the generation of species specific gravid trapping (OK).

We continued our phenological survey of the flowering plants of North Central FL. Naturally occurring, landscape and nursery plants were all included in this survey. Olfactometer studies using both male and female *Ae. albopictus* were conducted utilizing intact flowers of many different flowering plants. Positive responses were obtained from many of the plants, but the two which resulted in the best responses were the butterfly bush (*Buddleja davidi*) and golden rod (*Solidago* spp.). Additional olfactometer studies were conducted with these two plant types utilizing intact flowers, solvent extracts of whole flowers and individual compounds/blends that were isolated and identified by gas chromatography/mass spectrometry (ARS-Gainesville, FL).

Objective 5. Develop strategies for sustainable mosquito control by including training at all levels.

Andrea Egizi, graduate student, obtained an Ecology and Evolution Graduate Program Small Grants Award, Understanding the successful establishment of a temperate mosquito on a tropical island and received a Buell Award, Department of Ecology, Evolution and Natural Resources Opening the black box: Microbial metagenomics of larval mosquito feeding. George Condon, graduate student, started in September 2011 and submitted an NSF Graduate Research Fellowship entitled "High resolution spatial and temporal population genetics of invasive species". Rafael Valentin, undergraduate student will start as a graduate student in the fall of 2012. He is a co-author on a paper using qPCR for high throughput container *Aedes* egg identification. Rebekah Heiry, undergraduate student has been working with Dr. Fonseca since June 2011. She is now a student technician. Talks by Dr. Fonseca outside of the scientific meeting circuit aimed at enhancing understanding of vector-borne diseases and mosquito control. May, 2011: Cervia, Ravenna, Italy, Meeting entitled "Emerging vector borne disease: the role of *Aedes* mosquitoes". Talk entitled "The Asian tiger mosquito in the United States: past, present, and future". May 17 and May 25, 2011: two 3-hour presentations entitled "Enlightened mosquito control" to the Rutgers Environmental Stewards, at the Atlantic County Utility Authority, NJ and

the Duke Farms, Hillsborough, NJ. August, 2011: presented two talks in Ghana, Africa during the workshop on Genetics and Diseases Control (NJ).

Dr. Sebastien Marcombe, a postdoc funded by the Area-wide management of the Asian tiger mosquito, *Ae. albopictus* started May 2011 and has uncovered extensive patterns of insecticide resistance and reduced susceptibility in populations of *Ae. albopictus* (NJ).

Dr. Jiawu Xu funded in part by AW-ATM and in part by a new NIH-R21, has optimized 8 microsatellite loci for the Asian tiger mosquito. The NextGen sequencing project of *Ae. albopictus* to develop a high resolution SNP array is progressing, now funded by an NIH R21 (NJ).

One masters student was finished in OK in 2011, Ms. Kelsey Paras. Ms. Paras is continuing her education at the Ohio State University, pursuing a DVM. Dr. Valerie OBrien continued her post-doctoral position in mosquito ecology during 2011 (OK).

We co-instructed an all-day short course with Chris Lesser (Manatee County, FL, Mosquito Abatement District) on The Asian Tiger Mosquito: Biology & Control, at the Florida Mosquito Control Associations Dodd Short Courses (USDA-ARS, Gainesville, FL).

**Impact Statements:**

1. Work carried out in 2011 as part of the Northeast Regional Research Project, NE-1043, provided valuable theoretical and practical knowledge to help manage mosquito-borne disease.
2. New insights were obtained on the manner in which mosquito-borne viruses overwinter, persist and circulate in mosquito populations in the northeastern US.
3. Research resulted in a new understanding of the host selection patterns and genetic structure of *Culex pipiens* populations the primary vector mosquito species for West Nile virus.
4. New biological control agents were discovered and characterized using molecular methods, and existing biologic agents were evaluated in a variety of mosquito breeding habitats.
5. Empirically informed transmission models were developed for predicting West Nile virus foci on the basis of mosquito feeding, variation in local bird species availability, landscape heterogeneity, and local temperature and precipitation.
6. A climate-based mosquito stochastic and epidemiological model was developed to evaluate the epidemic potential of Chikungunya virus in the event of an introduction into the US.
7. The ecology, behavior, and genetic structure of two invasive mosquitoes, *Aedes albopictus* and *Aedes japonicus* were studied. Understanding these ecological relationships is essential to developing economical and effective integrated pest management programs for their control.
8. New methods for mosquito trapping in the field and virus isolation and identification in the laboratory are being evaluated to enhance mosquito and arbovirus surveillance programs.

9. Members are actively participating in graduate student and postdoctoral training programs in vector biology and mosquito and reporting research findings within the scientific community and with the general public.